The Action of Radium and certain other Salts on Gelatin.

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[Plate 7.]

The action of radio-active substances on gelatin media has recently attracted attention. In 'Nature'* there appears a letter by J. B. Burke, in which the writer states that certain "bacterial-like" cells are obtained as the result of the action, the cells grow up to a certain stage and subdivide, they contain a nucleus, and appear to be highly organised bodies. The author has made numerous experiments on this subject, and has made several communications to 'Nature' and to the Cambridge Philosophical Society.

The present paper deals chiefly with the results obtained by the aid of photography, which obviously is a much more satisfactory method of recording than mere drawing.

The word "growth" is used to indicate the action, but must not be taken to imply that anything of the nature of living growth has occurred.

Two methods of working were employed: (1) for unaided eye observation; (2) for microscopic examination.

In the first method small Soyka flasks with parallel sides were employed to hold the gelatin culture medium. The flask was about half filled with the gelatin and sterilised in the usual way by steaming. A few specks of the radium salt were then placed on the surface of the gelatin and the effect watched. At once a whitish patch was seen to develop round the speck, the patch rapidly increasing in size, so that at the end of 10 minutes it appeared like a growth of ordinary mould.

Plate 7, fig. 1A, represents the appearance of the growth at the end of about an hour. The patch continued to grow, but at a much slower rate, and in some cases reached a maximum in less than a day, the time required depending upon the stiffness of the jelly and upon the class of gelatin used. Several samples of radium salts of different degrees of purity were used, and it was seen that the rate and amount of growth did not depend upon the amount of radium present in the sample. Solutions of the salts were sometimes used, but the solid material was more satisfactory; the general

* Vol. 72, p. 78.

result was, however, the same. As radium salts are composed in the main part of barium salts, it seemed likely that the latter might have some effect upon the gelatin, and this was found to be the case, a growth being produced which seemed identical with that produced by the radium salt (fig. 1B). Fig. 2 shows the extent to which the growth had penetrated after 36 hours, and after which it ceased to increase.

A systematic examination was next made with all kinds of metallic salt, with the result that only those of strontium and lead, besides radium and barium, gave any decided effect. As these metals are those which form insoluble sulphates, it seemed likely that the growth originated about the precipitates which form with the sulphur compounds present in the gelatin.

As the use of the gelatin in the culture material is simply for the purpose of localising the growth, by keeping it more or less fixed, the effect of using other glutinous or mucilaginous substances instead of gelatin was tried. The substances thus employed were agar-agar and several varieties of starch and gums, sufficient being added to the meat solution to cause it to solidify. When this was done it was found that if *distilled* water was used in making up the jelly, *no growth could be seen*, but if *tap* water was used a slight growth made its appearance, and if a soluble sulphate was previously added, then a *very dense* growth appeared. It thus was quite evident that the presence of a sulphate was necessary for the formation of the growth, and attention was then directed to the gelatin to ascertain whether sulphuric acid or a sulphate was usually present. Between 30 and 40 samples of gelatin were examined and, with three exceptions, all contained sufficient sulphuric acid to give a distinct, sometimes a dense, precipitate with barium chloride in presence of nitric acid.* The gelatin solutions were prepared by washing the sample six times with distilled water, allowing it to soak for some time before decanting, then dissolving in sufficient distilled water to make a thin jelly.

Two samples of commercial Russian isinglass and one sample of gelatin prepared by the author from fresh calf skin failed to give a precipitate with barium or radium salts, but in each of these cases a growth could be obtained by adding a soluble sulphate. If a barium salt gave no precipitate with the gelatin, then radium salt failed to produce a growth. It thus seemed clear that the growth originated about the precipitate of barium sulphate.

The growth continues to extend for some time into the gelatin, but after a while it stops. The reason for this extension of the growth from the point of contact appears to be as follows: the gelatin allows of a slow diffusion of the

* The precipitate was analysed in several cases, and was found to consist of $BaSO_4$.

barium salt through it, and as there is only a small amount of sulphuric acid present the barium is in excess and, therefore, after forming a precipitate in the first layers of the gelatin, sufficient barium salt is left, which, diffusing onwards, causes further precipitation in the succeeding layers, but eventually all the barium salt is used up by combining with the sulphuric acid and then the growth reaches its limit.

A proof of this theory is given by the fact that, with samples of gelatin containing a (relatively) small amount of sulphuric acid, the growth, although much less dense, extends for a greater distance.

Figs. 3 and 4 are photographs of two flasks containing very pure gelatin. To 3 there was added a small amount of calcium sulphate solution, to 4 twice the amount was added. The growth can be seen to extend much further into the gelatin in 3 than in 4, although it is much less dense. Many observations confirm this.

As the experiments conducted in the flask did not admit of the action being watched from the beginning, another method was adopted which allowed of continuous observations being made. This method consisted in placing a little of the melted jelly upon a glass slip and, after allowing it to solidify, adding a speck of the salt whose action was to be studied and then covering with a thin glass circle. A modification of this plan was to place the cover glass upon the still liquid gelatin and allow it to solidify under the cover. A speck of the salt was then placed at the edge of the cover glass, and the growth worked its way through the thin layer of gelatin enclosed between the glasses. Many hundreds of preparations were made in this way, using all kinds of salts, and the results obtained have been the same as in the flask experiments, viz., that radium, barium, strontium, and lead salts are the only ones which produce any effect. It might be expected that calcium salt should behave in the same way as barium and strontium, but the calcium sulphate is so much more soluble than the sulphates of the other two metals that it does not form a precipitate under the conditions of the experiments.

Very careful search was made in the case of uranium salts, but not any growth could be obtained, negative results also following the use of thorium salts, pitchblende, and uranium metal. There is thus no connection between radio-activity and the formation of the growth.

A number of photographs were taken of the preparation made by the latter method at periods from a few minutes after contact with the gelatin to several days and in some cases weeks.

The apparatus used for this purpose was supplied by Zeiss, and for direct observation magnifying powers up to 1500 diameters could be employed.

For photography and projection magnification up to 6000 or 7000 diameters could be obtained without undue distortion. Photographs were taken with magnifications of 400, 1000, and 4000.

The first effect of the action of radium salt was to cause an evolution of gas in the form of minute bubbles, owing to the decomposition of the water; the evolution soon ceased, but simultaneously a nebulous growth was seen to proceed from the point of contact of the salt with the gelatin. The growth consisted of tiny particles of precipitate which increased in size rapidly up to a certain point, and then expanded much more slowly, and in many cases did not increase at all after 10 hours. This precipitate has, undoubtedly, a sort of cellular structure, as is clearly shown in the photographs taken with the higher powers.

Figs. 4 to 8 show the progressive stages of the growth during 85 minutes, the magnification being 400 diameters. Many "pairs" of cells can here be seen, but the grouping is purely fortuitous.

Fig. 9 represents a portion of the same preparation taken with a magnification of 1200 diameters. Here the cellular character is clearly seen. This same preparation was used to see whether any increase of size took place after one day.

For this purpose the slide was fixed firmly to the stage of the microscope, and the objective, 1/12 oil immersion, focussed upon a large cell which had a well-defined shape. This was photographed at intervals of a day for four successive days in order to determine whether there was anything of the nature of "cell division" or growth, in the usual sense, taking place (figs. 10 to 13).

The photographs absolutely negative this idea. Observation could not be carried on for a longer time on account of the drying of the immersion oil. It is important to observe that there is no *trace of a nucleus*, even on pushing the magnifying power by projection up to 12,000!!, this figure being, of course, a long way past the limit of "useful" magnifications.

A series of photographs was taken with barium salts instead of radium, with the results shown in figs. 14 to 17.

Fig. 14 shows the nature of the growth after 15 minutes at 400 diameters, fig. 15 after 30 minutes, fig. 16 the appearance at 1200 after one hour, and fig. 17 at 4000. It appears that there is not much difference between the result of the radium and barium salts; in fact, it is often impossible to say which metal has caused a particular growth. There is a considerable variation in the effect of both radium and barium salts, owing to the varying nature of the different samples of gelatin, and to the amount of water present, etc.

Fig. 18 shows the result at 4000 diameters of the action of strontium nitrate, and fig. 19 that due to lead nitrate upon the gelatin.

If these experiments are conducted upon gelatin from which the sulphuric acid has been removed no growths are obtained. A sample of gelatin from which the sulphuric acid had been removed was sealed up with some radium salt in September last, and at the present time no signs of growth have made their appearance, but if to a portion of the gelatin a soluble sulphate is added a growth at once appears.

It thus seems to be quite clear that the cellular growth cannot be produced by radium or barium unless a sulphate is present, and other metals, save Sr and Pb, fail to produce any result, because they do not form insoluble sulphates.

The cellular form of these precipitates is probably due to the circumstance that the gelatin is liquefied by the actions of the salt, and each particle of precipitate is formed about a core of gelatin, so that the layer of barium sulphate forms a kind of sac or cell which is surrounded by the solutions of the salt in the liquefied gelatin. This *cell* may be permeable to the liquefied gelatin containing a salt in solution, which, passing through the cell wall, causes an expansion to take place, the limit of growth being controlled by some surface tension effect.

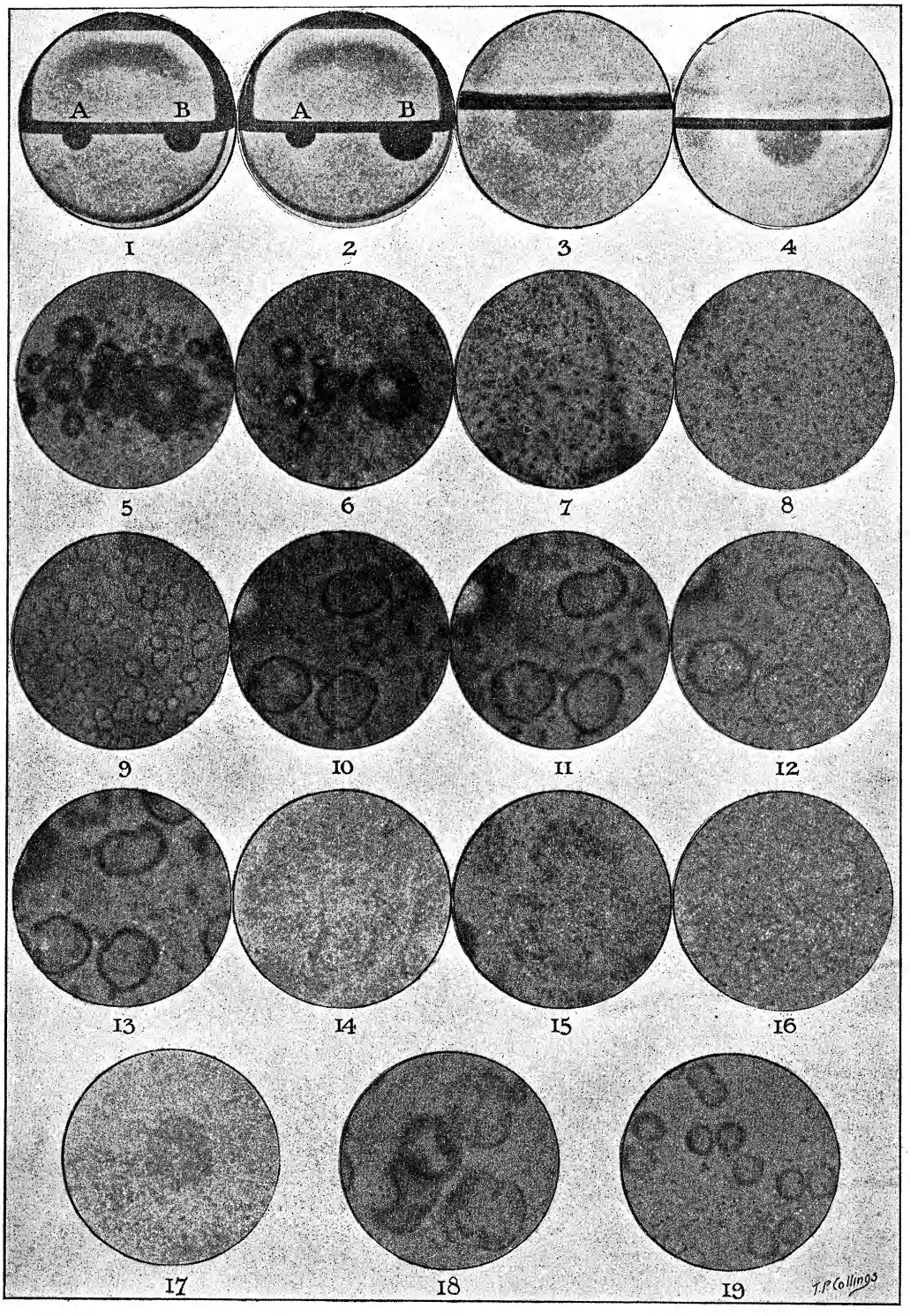
The conclusions which are drawn from a study of the photographs and direct examination under the microscope with high powers are that:—

1. The cells form round a precipitate of an insoluble sulphate, and the energy of the growth of the cell depends upon the amount of sulphate present.

2. *Radium has no specific action in forming cells*, any effect produced being due to the barium associated with it, and the purer specimens of radium salts are less satisfactory as cell-formers than the impurer ones. Probably pure radium salt would have no action except that of causing an evolution of gas.

3. The cells do not divide or bud or show anything resembling "karyokinesis," their growth very quickly reaches a maximum, and they do not decay or split up, save as a consequence of the drying of the gelatin. If the cover glass is sealed down with cement, the cells have been observed to suffer no alteration in the course of four months.

4. Radio-active substances, unless they contain barium, do not give rise to the formation of cells.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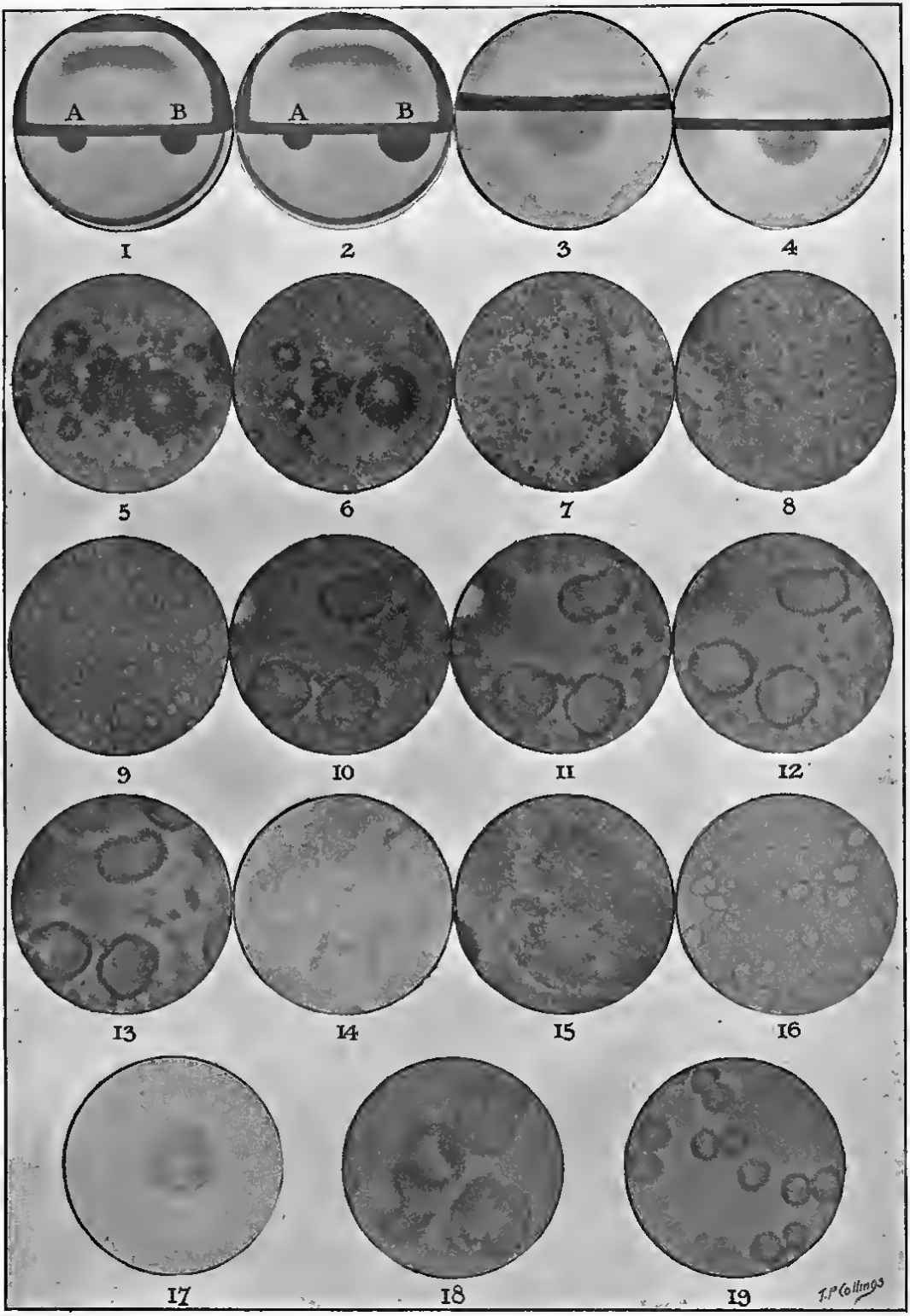
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